

1/23

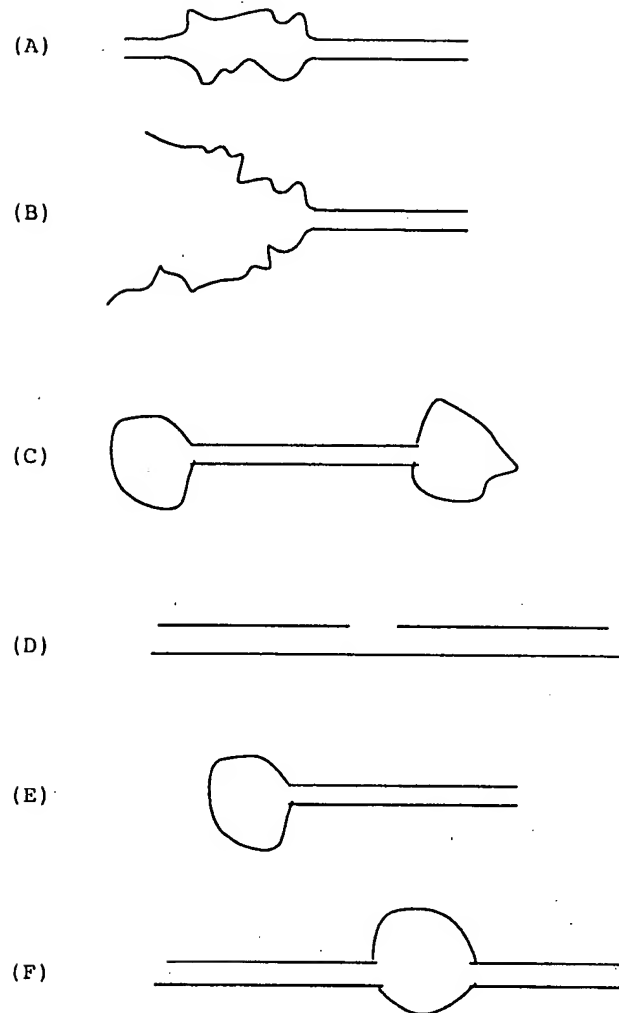


Figure 1 (A-F)

Construct Forms Comprising at Least one Single-Stranded Region

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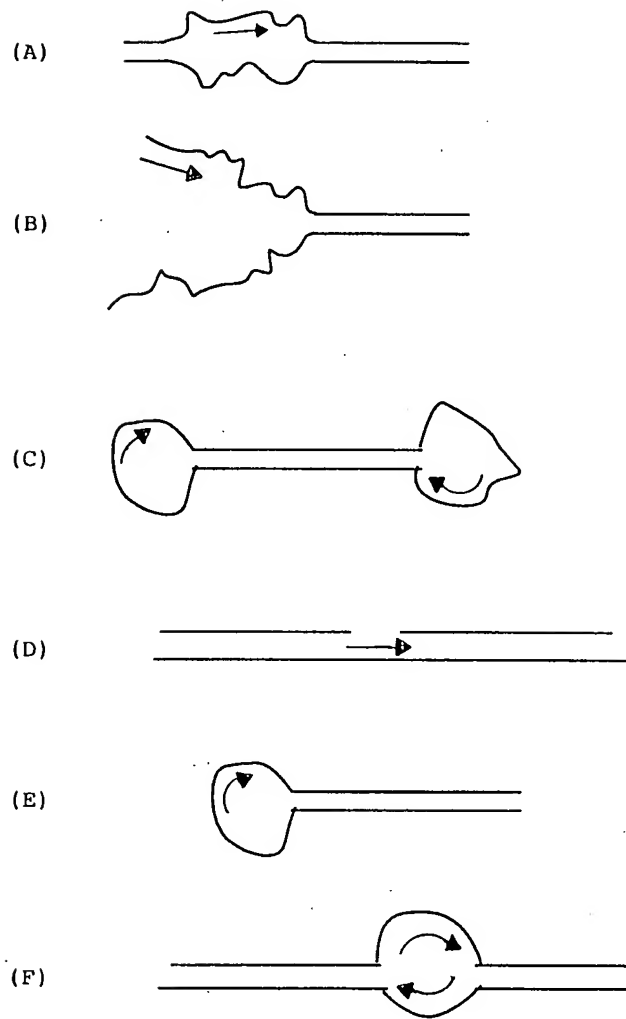


Figure 2 (A-F)

Functional Forms of the Construct

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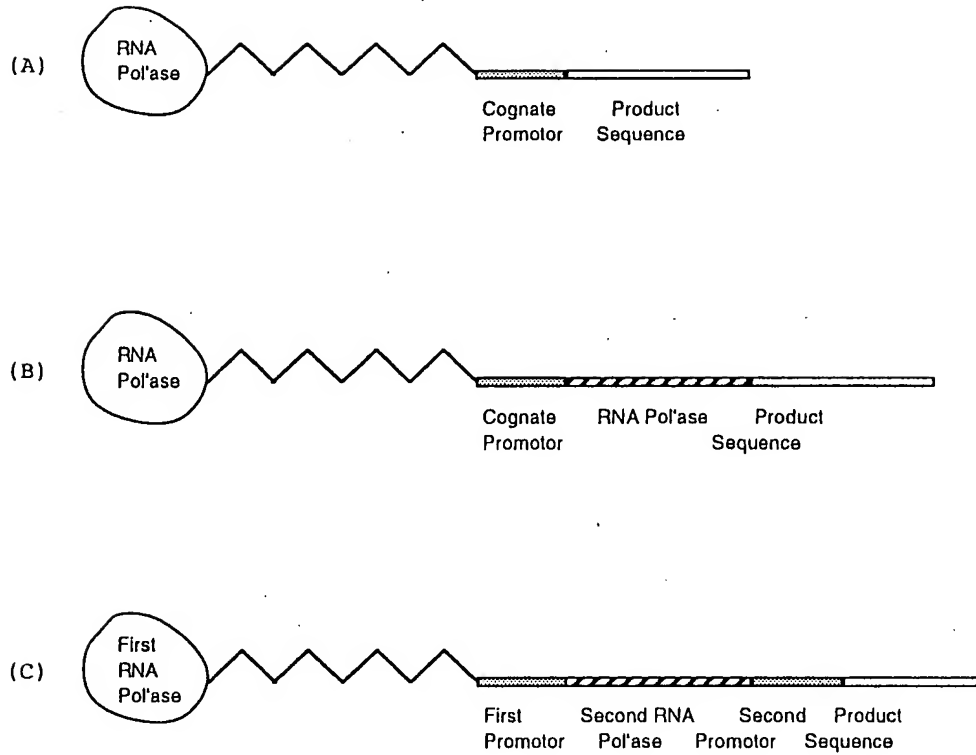


Figure 3 (A-C)

Three Constructs with an RNA Polymerase
Covalently Attached to a Transcribing Cassette

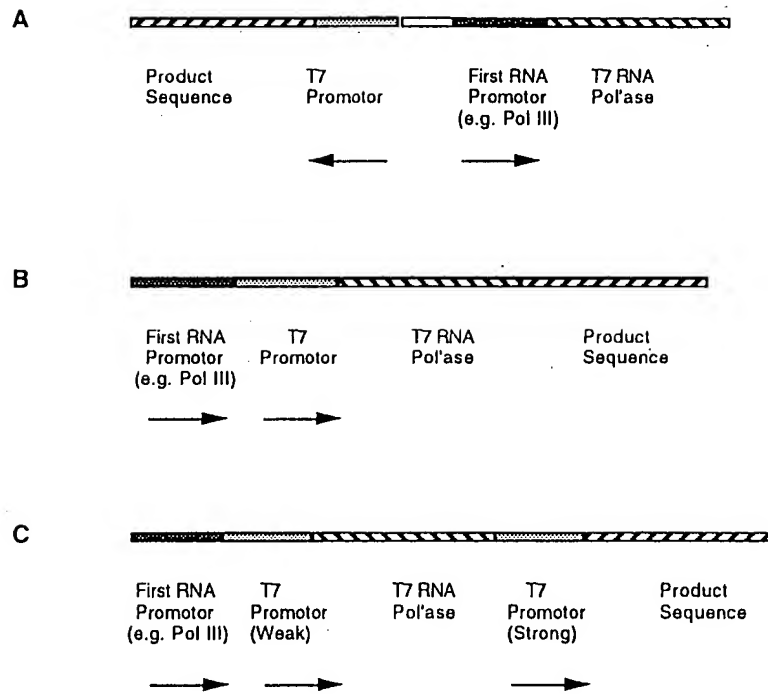


Figure 4 (A-C)

Three Constructs with Promoters
for Endogenous RNA Polymerase

M13mp18. Seq Length: 7250

1.	AATGCTACTA	CTATTAGTAG	AATTGATGOC	AOCTTTTCAG	CTOGOGOOOC
51.	AAATGAAAAT	ATAGCTAAAC	AGGTTATTGA	OCATTTGOGA	AATGTATCTA
101.	ATGGTCAAAC	TAAATCTACT	CGTTGOCAGA	ATTGGGAATC	AACTGTTACA
151.	TGGAATGAAA	CTTCCAGACA	COGTACTTTA	GTTGCATATT	TAAAACATGT
201	TGAGCTACAG	CAOCAGATTC	AGCAATTAAG	CTCTAAGOCA	TOCGCAAAAA
251	TGAOCTCTTA	TCAAAAGGAG	CAATTAAAGG	TACTCTCTAA	TOCTGAOCTG
301.	TTGGAGTTTG	CTTCCGGTCT	GGTTGCTTTT	GAAGCTCGAA	TAAAAOOGOG
351.	ATATTTGAAG	TCTTTOGGGC	TTOCTCTTAA	TCTTTTTGAT	GCAATOOGCT
401.	TTGCTTCTGA	CTATAATAGT	CAGGGTAAAG	AOCTGATTTT	TGATTTATGG
451.	TCATTCTCGT	TTTCTGAACT	GTTTAAAGCA	TTTGAGGGGG	ATTCAATGAA
501.	TATTTATGAC	GATTOGOCAG	TATTGGAOGC	TATOCAGTCT	AAACATTTTA
551.	CTATTACOOO	CTCTGGCAAA	ACTTCTTTTG	CAAAGOCTC	TOGCTATTTT
601.	GGTTTTTATC	GTCGTCTGGT	AAOCGAGGGT	TATGATAGTG	TTGCTCTTAC
651.	TATGOCTOGT	AATTCCTTTT	GGCGTTATGT	ATCTGCATTA	GTTGAATGTG
701.	GTATTOCTAA	ATCTCAACTG	ATGAATCTTT	CTAOCTGTAA	TAATGTTGTT
751.	COGTTAGTTC	GTTTTATTAA	CGTAGATTTT	TCTTCCCAAC	GTOCTGACTG
801.	GTATAATGAG	CCAGTTCTTA	AAATGOCATA	AGGTAATTCA	CAATGATTAA
851.	AGTTGAAATT	AAOCATCTC	AAGCCCAATT	TACTACTOGT	TCTGGTGTTC
901.	TOGTCAGGGC	AAGCTTATT	CACTGAATGA	GCAGCTTTGT	TACGTTGATT
951.	TGGGTAATGA	ATATCOGGTT	CTTGTOGAAG	ATTACTCTTG	ATGAAGGTCA
1001	GOCAGOOTAT	GOGOOTGGTC	TGTACAOOGT	TCATCTGTGC	TCTTTCAAAG
1051	TTGGTCAGTT	CGGTTCCCTT	ATGATTGAOC	GTCTGOGOOT	CGTTCCGGCT
1101	AAGTAACATG	GAGCAGGTGC	CGGATTTGGA	CACAATTTAT	CAGGOGATGA
1151	TACAAATCTC	CGTTGTAOCTT	TGTTTGGGCG	TTGGTATAAT	CGCTGGGGGT
1201	CAAAGATGAG	TGTTTTAGTG	TATTCTTTGC	CCTCTTTCGT	TTTAGGTTGG

Figure 5

M13mp18 Nucleic Acid Sequence

1251	TGCTTGGTA	GTGGCATTAC	GTATTTTACC	OGTTTAATGG	AACTTCTCTC
1301	ATGAAAAAGT	CTTTAGTCCT	CAAAGCCTCT	GTAGCGGTG	CTAOCCTCGT
1351	TOCGATGCTG	TCTTTOGCTG	CTGAGGGTGA	OGATCOOGCA	AAAGOGGCOCT
1401	TTAACTCOCT	GCAAGCCTCA	GCGACCGAAT	ATATCGGTTA	TGCGTGGGGG
1451	ATGGTTGTTG	TCATTGTGGG	CGCAACTATC	GGTATCAAGC	TGTTTAAGAA
1501	ATTCACTCG	AAAGCAAGCT	GATAAACCGA	TACAATTAAA	GGCTCTTTT
1551	GGAGCCTTTT	TTTTTGAGA	TTTCAACGT	GAAAAAATTA	TTATTOGCAA
1601	TTCTTTAGT	TGTTCTTTC	TATTCTCACT	COGCTGAAAC	TGTTGAAAGT
1651	TGTTTAGCAA	AACCCATAC	AGAAAATCA	TTACTAACG	TCTGGAAGA
1701	CGACAAAAC	TTAGATCGTT	ACGCTAACTA	TGAGGGTTGT	CTGTGGAATG
1751	CTACAGGCGT	TGTAGTTTGT	ACTGGTGAOG	AACTCAGTG	TTACGGTACA
1801	TGGGTTOCTA	TTGGGCTTGC	TATCOCTGAA	AATGAGGGTG	GTGGCTCTGA
1851	GGGTGGGGT	TCTGAGGGTG	GCGTTTCTGA	GGGTGGGGT	ACTAAOCTC
1901	CTGAGTAOGG	TGATACAOC	ATTOGGGGCT	ATACTTATAT	CAOOCCTCTC
1951	GACGGCACTT	ATCOGCTGG	TACTGAGCAA	AACCGCTA	ATCTAATCC
2001	TTCTCTTGAG	GAGTCTCAGC	CTCTTAATAC	TTTCATGTTT	CAGAATAATA
2051	GGTTOGAAA	TAGGCAGGGG	GCATTAAC	TTTATAOGGC	CACTGTTACT
2101	CAAGGCACTG	AACCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGOCATG	TATGACGCTT	ACTGGAACGG	TAAATTCAGA	GACTGCGCTT
2201	CAAGGCACTG	AACCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGOCATG	TGCTCAAOC	TOCTGTCAAT	GCTGGGGGGG	GCTCTGGTGG
2201	TOCATTTCTGG	CTTTAATCAA	GATOCATTGG	TTTGTGAATA	TCAAGGOCOA
2251	TGCTCTGAOC	TGCTCAAOC	TOCTGTCAAT	GCTGGGGGGG	GCTCTGGTGG
2301	TGGTTCTGGT	GGGGCTCTG	AGGGTGGTGG	CTCTGAGGGT	GGGGTTCTG
2351	AGGGTGGGG	CTCTGAGGGA	GGGGTTTOOG	GTGGTGGCTC	TGGTTTOGGT
2401	GATTTTGATT	ATGAAAAGAT	GGCAAAOGCT	AATAAGGGGG	CTATGAOOGA
2451	AAATGCOGAT	GAAAAOGGG	TACAGTCTGA	CGCTAAAGGC	AACTTGATT

Figure 5

M13mp18 Nucleic Acid Sequence

2501	CTGTGCTAC	TGATTACGGT	GCTGCTATCG	ATGGTTTCAT	TGGTGAOGTT
2551	TOCGGOCCTG	CTAATGGTAA	TGGTGCTACT	GGTGATTTTG	CTGGCTCTAA
2601	TTCCCAAATG	GCTCAAGTOG	GTGAOGGTGA	TAATTCACCT	TTAATGAATA
2651	ATTTCOGTCA	ATATTTACCT	TOOCTOOCCTC	AATOGGTTGA	ATGTGGOOCT
2701	TTTGCTTTTA	GOGCTGGTAA	AOCATATGAA	TTTTCTATTG	ATTGTGACAA
2751	AATAAACTTA	TTOOGTGGTG	TCTTTGCGTT	TCTTTTATAT	GTTGOCACCT
2801	TTATGTATGT	ATTTTCTACG	TTTGCTAACA	TACTGCGTAA	TAAGGAGTCT
2851	TTATCATGCC	AGTTCCTTTG	GGTATTCOGT	TATTATTGCG	TTTOCTCGGT
2901	TTCTTCTGG	TAACTTTGTT	CGGCTATCTG	CTTACTTTTC	TTAAAAAGGG
2951	CTTGGSTAAG	ATAGCTATTG	CTATTTCAAT	GTTTCTTGCT	CTTATTATTG
3001	GGCTTAACTC	AATTCTTGTTG	GGTTATCTCT	CTGATATTAG	CGCTCAATTA
3051	COCTCTGACT	TTGTTCAAGG	TGTTCAAGTTA	ATTCTCOOCT	CTAATGOGCT
3101	TCOCTGTTTT	TATGTTATTC	TCTCTGTAAA	GGCTGCTATT	TTCAATTTTG
3151	ACGTAAACA	AAAAATCGTT	TCTTATTTGG	ATTGGGATAA	ATAATATGGC
3201	TGTTTTATTT	GTAACCTGGCA	AATTAGGCTC	TGGAAAGAOG	CTOGTTAGOG
3251	TTGGTAAGAT	TCAGGATAAA	ATTGTAGCTG	GGTGCAAAAT	AGCAACTAAT
3301	CTTGATTTAA	GGCTTCAAAA	OCTOOCGCAA	GTOGGGAGGT	TOGCTAAAAC
3351	GCTOGOGTT	CTTAGAATAC	OGGATAAGOC	TTCTATATCT	GATTTGCTTG
3401	CTATTGGGOG	OGGTAATGAT	TOCTACGAATG	AAAATAAAAA	CGGCTTGCTT
3451	GTTCTOGATG	AGTGOGGTAC	TTGGTTTAAT	AOCOCTTCTT	GGAATGATAA
3501	GGAAAGACAG	COGATTATTG	ATTGGTTTCT	ACTGCTCGT	AAATTAGGAT
3551	GGGATATTAT	TTTTCTTGTT	CAGGACTTAT	CTATTGTTGA	TAAACAGGOG
3601	OGTTCTGCAT	TAGCTGAACA	TGTTGTTTAT	TGTOGTGTC	TGGACAGAAT
3651	TACTTTAOCCT	TTTGTOGGTA	CTTTATATTC	TCTTATTAAT	GGCTOGAAAA
3701	TGCTCTGOC	TAAATTACAT	GTTGGOGTTG	TTAAATATGG	OGATTCTCAA
3751	TTAAGOOCTA	CTGTTGAGOG	TTGGCTTTAT	ACTGGTAAGA	ATTTGTATAA
3801	CGCATATGAT	ACTAACAGG	CTTTTCTAG	TAATTATGAT	TCOGGTGTTT

Figure 5

M13mp18 Nucleic Acid Sequence

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3851 ATTCTTATTT AAGGCTTAT TTATCACACG GTOGGTATTT CAAACCATTA
3901 AATTTAGGTC AGAAGATGAA ATTAACATAA ATAATATTGA AAAAGTTTTT
3951 TCGGTTCTTT TGTCTTGCGA TTGGATTTCG ATCAGCATTT ACATATAGTT
4001 ATATAACCCA AOCTAAGGCG GAGGTTAAAA AGGTAGTCTC TCAGAOCTAT
4051 GATTTTGATA AATTCATCTAT TGAATCTTCT CAGGCTCTTA ATCTAAGCTA
4101 TCGCTATGTT TTCAAGGATT CTAAGGGAAA ATTAATTAAT AGOGAOGATT
4151 TACAGAAGCA AGGTTATTCA CTCACATATA TTGATTTATG TACTGTTTCC
4201 ATTAATAAAG GTAATTCAAA TGAAATTGTT AAATGTAATT AATTTTGTTT
4251 TCTTGATGTT TGTTCATCA TCTTCTTTTG CTCAGGTAAT TGAAATGAAT
4301 AATTCGGCTC TCGGCGATTT TGTAACCTGG TATTCAAAGC AATCAGGCGA
4351 AATCCGTTATT GTTCTCTCCG ATGTAAAAGG TACTGTTACT GTATATTCAT
4401 CTGAGGTTAA AOCTGAAAAT CTAOGCAATT TCTTTATTTT TGTTTTAOGT
4451 GCTAATAATT TTGATAATGGT TGGTTCAATT CCTTCATAA TTCAGAAGTA
4501 TAATCCAAAC AATCAGGATT ATATTGATGA ATTGOCATCA TCTGATAATC
4551 AGGAATATGA TGATAATTCC GCTCCTTCTG GTGGTTTCTT TGTTCGCAA
4601 AATGATAATG TTAATCAAAC TTTTAAATTT AATAAGTTT GGGCAAAGGA
4651 TTAATAACGA GTTGTOGAAT TGTTTGTAAG GTCTAATACT TCTAAATCCT
4701 CAAATGTATT ATCTATTGAC GGCTCTAATC TATTAGTTGT TAGTGCTCCT
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4801 AACTGAOCAG ATATTGATTG AGGGTTTGAT ATTTGAGGTT CAGCAAGGTG
4851 ATGCTTTAGA TTTTTCATTT GCTGCTGGCT CTCAGGTTGG CACTGTTGCA
4901 GGGGGTGTTA ATACTGAOOG CCTCAOCTCT GTTTTATCTT CTGCTGGTGG
4951 TCGTTTGGT ATTTTAAATG GCGATGTTTT AGGGCTATCA GTTCGGCAT
5001 TAAAGACTAA TAGOCATTCA AAAATATTGT CTGTGOCACG TATTCTTACG
5051 CTTTCAGGTC AGAAGGGTTC TATCTCTGTT GGOCAGAATG TCCCTTTTAT
5101 TAAAGACTAA TAGOCATTCA AAAATATTGT CTGTGOCACG TATTCTTACG
5151 CGATTGAGCG TCAAAATGTA GGTATTTCCA TGAGCGTTTT TCCTGTTGCA

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Figure 5

M13mp18 Nucleic Acid Sequence


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5201 ATGGCTGGGG GTAATATTGT TCTGGATATT AOCAGCAAGG OOGATAGTTT
5251 GAGTTCCTCT ACTCAGGCAA GTGATGTTAT TACTAATCAA AGAAGTATTG
5301 CTACAAOOGT TAATTTGOGT GATGGACAGA CTCTTTTACT OGGTGGOOCTC
5351 ACTGATTATA AAAACACTTC TCAAGATTCT GGOGTAOOGT TOCTGTCTAA
5401 AATOOCTTTA ATOGGOCTOC TGTTTAGCTC OOGCTCTGAT TOCAAOGAGG
5451 AAAGCAOGTT ATACGTGCTC GTCAAAGCAA OCATAGTAOG OGOOCTGTAG
5501 OGGOGCATT AOGOGGGOGG GTGTGGTGGT TAOGOGCAGC GTGAOOGCTA
5551 CACTTGOCAG OGOOCTAGOG OOOGCTOCTT TCGCTTTCTT COCTTCTTTT
5601 CTGOCACOGT TOGOOGGCTT TCOOOGTCAA GCTCTAAATC GGGGGCTOOC
5651 TTTAGGGTTC OGATTTAGTG CTTTACGGCA OCTOGAOCOC AAAAACTTG
5701 ATTTGGGTGA TGGTTCACGT AGTGGGOCAT OGOOCTGATA GACGGTTTTT
5751 OGOOCTTTGA OGTTGGAGTC CAOGTTCTTT AATAGTGGAC TCTTGTTOCA
5801 AACTGGAACA ACACTCAOOC CTATCTOGGG CTATTCTTTT GATTTATAAG
5851 GGATTTTGOC GATTTGGGAA OCACCATCAA ACAGGATTTT OGOCTGCTGG
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6151 TTATGCTTCC GGCTOGTATG TTGTGTGGAA TTGTGAGOGG ATAACAATTT
6201 CACACAGGAA ACAGCTATGA CCATGATTAC GAATTCGAGC TOGGTAOOOG
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6351 OCTTGACGCA CAATOOOCTT TOGOCAGCTG GOGTAATAGC GAAGAGBOOC
6401 GCACOGATOG COCTTCCCAA CAGTTGOGCA GOCTGAATGG OGAATGGOGC
6451 TTTGCTGGT TTOGGCAOC AGAAGOGGTG OOGGAAAGCT GGCTGGAGTG
6501 OGATCTTOCT GAGGBOGATA OGGTGTGTGT OOOCTCAAAC TGGCAGATGC

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Figure 5

M13mp18 Nucleic Acid Sequence

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6551	ACGGTTAOGA	TGOGGOCATC	TACACCAAOG	TAAOCTATOC	CATTACGGTC
6601	AATCOGGOGT	TTGTTCCAC	GGAGAATCOG	ACGGGTGTGT	ACTOGCTCAC
6651	ATTTAATGTT	GATGAAAGCT	GGCTACAGGA	AGGOCAGAOG	CGAATTATTT
6701	TTGATGGOGT	TCCTATTGGT	TAAAAAATGA	GCTGATTTAA	CAAAAATTTA
6751	ACGCGAATTT	TAACAAAATA	TTAACGTTTA	CAATTTAAAT	ATTTGCTTAT
6801	ACAATCTTCC	TGTTTTTGGG	GCTTTTCTGA	TTATCAACCG	GGGTACATAT
6851	GATTGACATG	CTAGTTTTAC	GATTACOGTT	CATCGATTCT	CTTGTTTGCT
6901	CCAGACTCTC	AGGCAATGAC	CTGATAGOOT	TTGTAGATCT	CTCAAAAATA
6951	GCTACOCCTCT	COGGCATGAA	TTTATCAGCT	AGAACGGTTG	AATATCATAT
7001	TGATGGTGAT	TTGACTGTCT	COGGOCCTTC	TCACOCCTTTT	GAATCTTTAC
7051	CTACACATTA	CTCAGGCATT	GCATTTAAAA	TATATGAGGG	TTCTAAAAAT
7101	TTTTATCCTT	GCGTTGAAAT	AAAGGCTTCT	COOGCAAAAG	TATTACAGGG
7151	TCATAATGTT	TTTGGTACAA	COGATTTAGC	TTTATGCTCT	GAGGCTTTAT

Figure 5

M13mp18 Nucleic Acid Sequence

COMPLEMENTARY TO M₁₃

POSITION	5' . . . 3'	POSITION	
645	AGCAACACTATCATA	631	M ₁₃ /1
615	ACGACGATAAAAAAC	601	M ₁₃ /2
585	TTTTGC AAAAGAAGT	571	M ₁₃ /3
555	AATAGT AAAATGTTT	541	M ₁₃ /4
525	CAATACTGCGGAATG	511	M ₁₃ /5
495	TGAATCCCCCTCAAA	481	M ₁₃ /6
465	AGAAAACGAGAATGA	451	M ₁₃ /7
435	CAGGTCTTTAOCCTG	421	M ₁₃ /8
405	AGGAAAGCGGATTGC	391	M ₁₃ /9
375	AGGAAGCCCGAAAGA	361	M ₁₃ /10

COMPLEMENTARY TO SS PHAGE DNA

POSITION	5' . . . 3'	POSITION	
351	ATATTTGAAGTCTTT	366	M ₁₃ /11
371	TCTTTTGTGCAAT	386	M ₁₃ /12
391	CTATAATACTCAGGG	406	M ₁₃ /13
411	TGATTATGGTCATT	426	M ₁₃ /14
431	GTTTAAAGCATTGTA	446	M ₁₃ /15
451	TATTTATGACGATTC	466	M ₁₃ /16
471	TATCCAGTCTAAACA	486	M ₁₃ /17
491	CTCTGGCAAACTTC	506	M ₁₃ /18
511	TCGCTATTTTGGTTT	526	M ₁₃ /19
531	AAACGAGGGTTATGA	546	M ₁₃ /20

Figure 6

Primers for Nucleic Acid Production
Derived from M13mp18 Sequence

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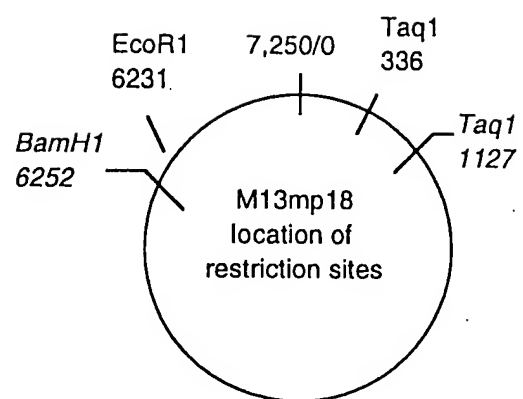
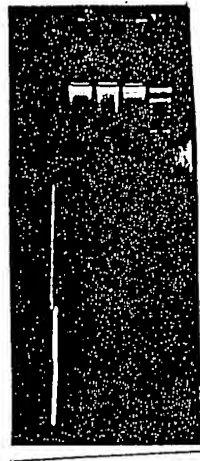


Figure 7

Appropriate M13mp18 Restriction Sites

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Lane 1: from calf thymus + Taq digested mp18 amplification reaction
Lane 2: from Taq digested mp18 amplification reaction
Lane 3: from calf thymus amplification reaction
Lane 4: øX174 Hinf1 size marker

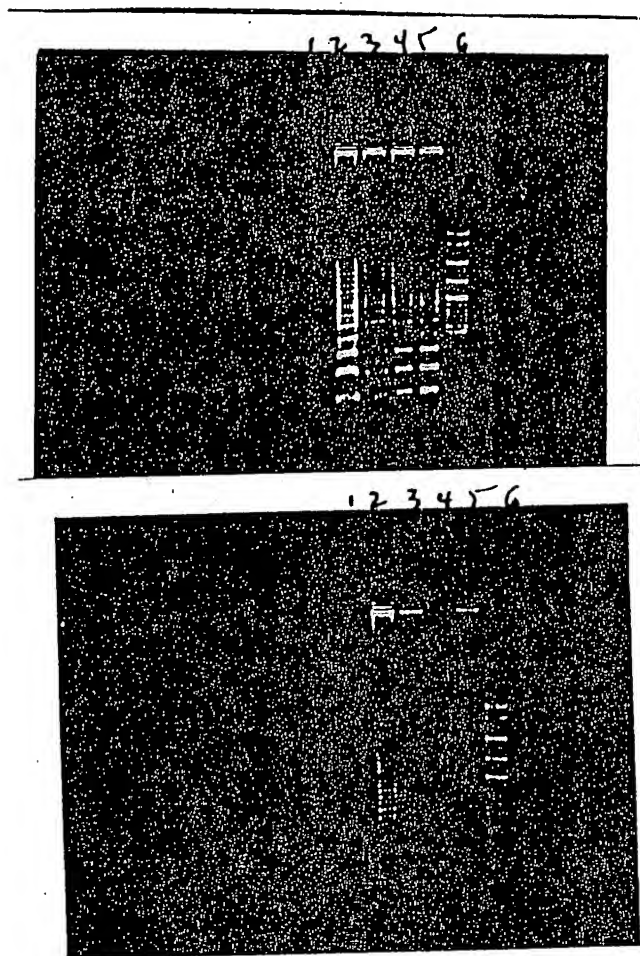
Figure 8

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Lane 1: no template
Lane 2: mp18 template, phosphate buffer
Lane 3: MspI/pBR322 size marker
Lane 4: mp18 template, MOPS buffer

Figure 9

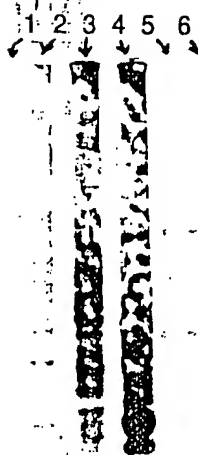


Top= (+) Template
Bottom= (-) Template

Lane 1: phosphate buffer
Lane 2: MES
Lane 3: MOPS
Lane 4: DMAB
Lane 5: DMG
Lane 6: pBR322/Mspl size marker

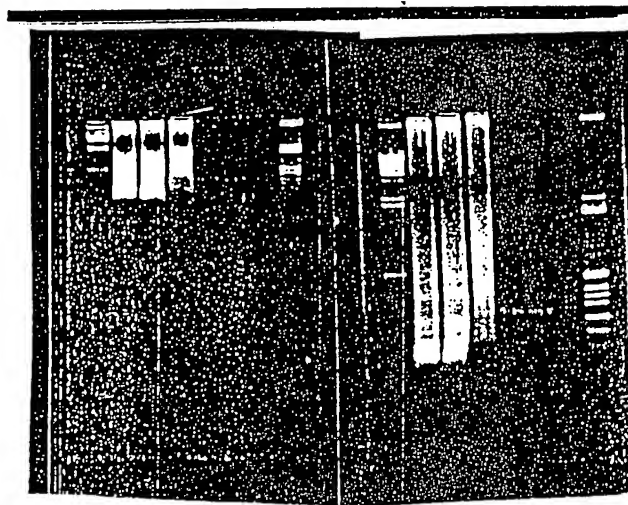
Figure 10

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Lane 1: DMAB buffer, no template
Lane 2: DMAB buffer, mp18 template
Lane 3: DMG buffer, no template
Lane 4: DMG buffer, mp18 template
Lane 5: No reaction
Lane 6: 200 ng Taq I digested mp18
size marker/positive control

Figure 11



First Time Interval Second Time Interval

Agarose Gel Analysis

- Lane 1: lambda Hind III marker
- Lane 2: Amp/Untreated
- Lane 3: Amp/Kinased
- Lane 4: Amp/Kinased/Ligated
- Lane 5: PCR/Untreated
- Lane 6: PCR/Kinased
- Lane 7: PCR/Kinased/Ligated
- Lane 8: øX174/Hinf1 marker

Figure 12

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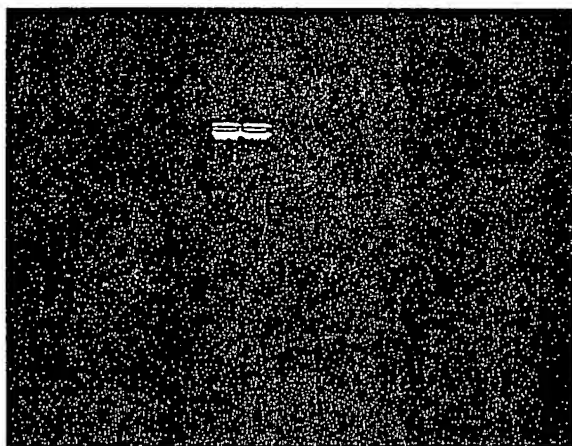
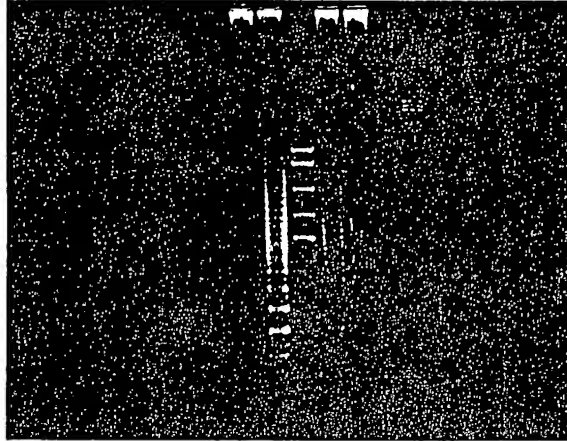


Figure 13

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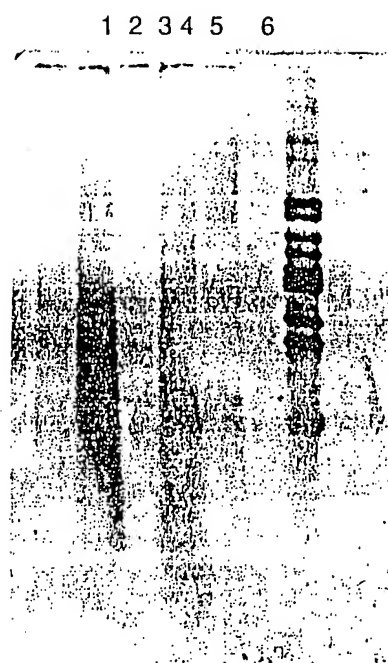
1 2 3 4 5 6



Lane 1: Primers alone
Lane 2: Primers + taq digested M13 DNA
Lane 3: Molecular weight markers
Lane 4: Primers + RNA
Lane 5: Primers alone
Lane 6: M13 digested DNA
Buffer was dimethyl amino glycine, pH 8.6

Figure 14

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Lane 1: Primers alone
Lane 2: Primers + taq digested M13 DNA
Lane 3: Molecular weight markers
Lane 4: Primers + RNA
Lane 5: Primers alone
Lane 6: M13 digested DNA
Buffer was dimethyl amino glycine, pH 8.6

Figure 15

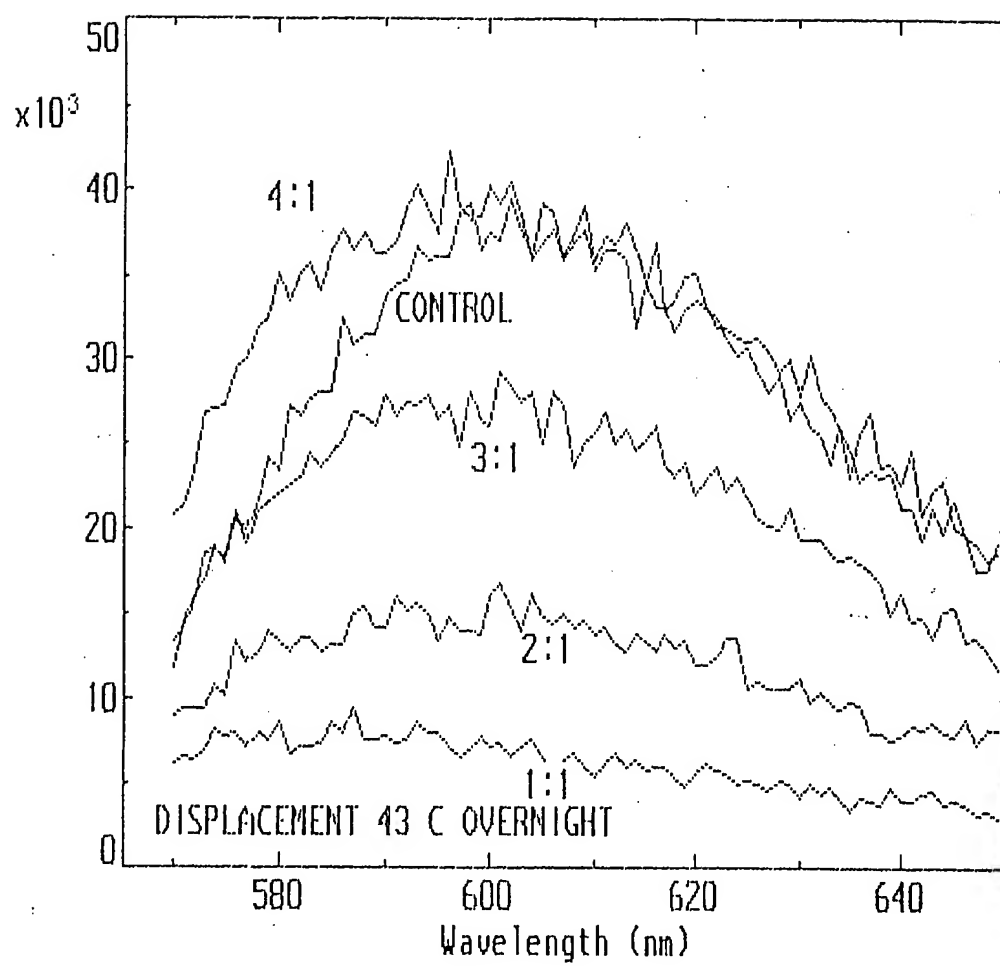


Figure 16

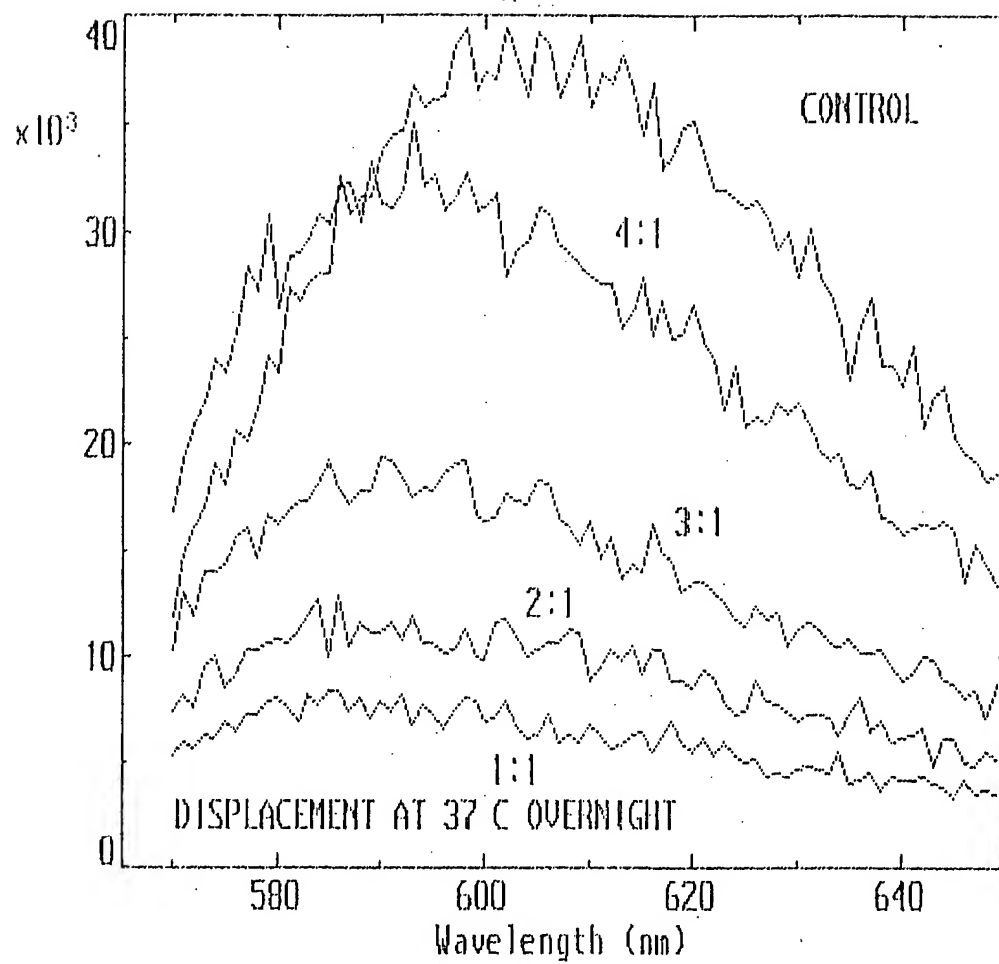


Figure 17

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pIBI 31-BH5-2

fmct AUG of Lac z (T7 Promotor region....
LAC PROMOTOR..ATG ACC ATG ATT ACG CCA GAT ATC AAA TTA ATA CGA CTC ACT ATA
oligo 50-mer 3'- tac t'aa t'gc ggt' ct'a t'ag t'Vt aat' tat' gct' gag t'ga t'at' c-5'
10 base insert
T7 RNA Start («« T3 Promotor Region)
IGGG CTC ICCT TTA GTG ACG GTT AAT
....») «- T3 Start Signal

pIBI 31 BSII/HCV

fmct AUG of Lac z (T3 Promotor region -») T3 RNA Start
LAC PROMOTOR ..ATG ACC ATG ATT ACG CCA AGC TCG AAA TTA ACC CTC ACT AAA /GGG
oligo 50-mer 3'- tac t'aa t'ac t'aa t'gc ggt' t'V--10 base insert--.....
MULTIPLE CLONING SITE + 390 BASE INSERT CTA /TAG TGA GTC CGT ATT AAT....
«- T7 Promotor Region)
«- T7 Start Signal
5'-ct'a t'ag t'ga gt'c gt'a tt'a at'.....

Figure 18